DEVELOPMENT OF A HIGH DENSITY PERCUTANEOUS CONNECTOR SYSTEM

QUARTERLY REPORT #4
January 15, 1998 - April 15, 1998

N01-DC-7-2103

THIS QPR IS BEING SENT TO YOU BEFORE IT HAS BEEN REVIEWED BY THE STAFF OF THE NEURAL PROSTHESIS PROGRAM.

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Abstract

This report summarizes activity over the period from January 15, 1998 through April 15, 1998 on NIH Contract N01-DC-7-2103, "Development of a High Density Percutaneous Connector System". Two connectors were implanted at HMRI, one for electrostimulation of skin growth and one to test the effect of Ti beads on skin growth and the first flat cable at skull level. Meetings were held with researchers at the University of Washington (UWEB) who have developed a method of promoting skin attachment to inert materials such as Ti. Four ceramic materials were molded and machined into the shape of connectors with the results that one did not machine properly and others shrunk excessively when fired. Tooling for a CABAL-12 pin long-term implant matrix material has been completed with first twelve-pin dummy connectors expected soon. A search for polymers that might also be useful for long term implant continues with the fabrication of a hot box for accelerated life testing. Several designs for a quick disconnect mechanism are under consideration with the first design rejected. Details from the implants conducted at HMRI during Fall 1997 are attached as Appendix I.

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I. Background and Review of Contract Requirements

This report summarizes activity from July 15 through October 15, 1997, on NIH Contract N01-DC-7-2103, "Development of a High Density Percutaneous Connector System". Over the course of this contract, a high density, planar, low profile connector system is being developed that incorporates pad grid array technology. This technology has unique advantages as applied to a percutaneous interconnect system. In particular the connector system will be low in profile, easy to clean, sealed against ingress of contaminants, offer low mechanical resistance to mating and demating and provide a very high number of contacts in a small diameter. The connector system will be implanted in a suitable animal model and the appropriate electrical, mechanical, and biocompatible properties of the system will be assessed. The specific technical requirements of this connector system as detailed in the contract are explained below:

- The connector will incorporate a pedestal that can be attached to the skull in a mechanically stable manner. The pedestal will be designed to accept a replaceable connector assembly. All materials of the pedestal in contact with tissue will be biocompatible and the profile of the pedestal will be low enough to minimize any physical trauma during mating and demating of the connector or due to normal physical activities.

- The connector assembly will be high-density with at least 70 contacts. The electrical isolation between the contacts or between the contacts and the body should withstand at least 18 volts without breakdown. The connector contacts when mated should be capable of passing up to 20 mA of current with less than a 1.0 volt drop across the connection. A simple method of mating and demating the upper and lower surfaces of the connector should be provided. In addition, a convenient means to attach electrical leads to the connector is needed.

- The connector will be designed from materials that are durable and can withstand the physical abuse from normal activities of daily living. The interface between the connector and the skin must be such that the passage of microorganisms into the body and fluid drainage out of the body is prevented.

- In earlier studies connectors had 5 separate loops of insulated wire, each 2 inches long. Because of wire breakage observed during these studies it is necessary to make a more durable and a more realistic part. Future connectors will have only one flat "cable" 1 to 1.5 inches long with 10 Pt/Ir wires, each 1 mil in diameter, coated with Parylene and Silicone. The ends of the wires are welded so as to make 5 "loops" and the ends will be coated with Silicone. An 18 volt bias will be maintained on the connector contacts and insulated wires relative to an implanted platinum wire connected to one of the unused contacts. The leakage current of the wires will be monitored and if more than 10 nanoamperes of current is detected, the source of the leakage will be identified and corrected.

- Performance of the connector system will be tested in a suitable animal model. After six months of implantation, the connector assembly will be explanted and gross and microscopic examinations will be performed to study the attachment of the pedestal to the skull, the attachment of the skin and soft tissue surrounding the pedestal to the pedestal wall and the reaction of adjacent tissue to the implanted device.

- Finally, design changes and improvements, if needed, will be recommended. A set of connectors will be fabricated and sent to the NIH for implantation into primates and eventually humans as part of their ongoing research.

II. HMRI Work

Two implants have been performed at HMRI. The first studied the electrostimulation of skin growth and the second was the first flat skull level cable implant intended to fix wire breakage reported in earlier implants. The second implant also had Ti beads around half the ring to study skin growth into the beads. The other half of the surface was relatively smooth. Both implants continued studies of ossiointegration.

The first implant to study electrostimulation failed because the connector was damaged when bumped on the cage by the cat. The configuration of this dummy connector was a split solid "plug" in place of the bottom connector section. The two halves were epoxied back together with a Pt/Ir wire between them. The epoxy insulated the two connector halves. The wire was coated with Silicone as an insulator. An 0.4 inch length of uninsulated wire was placed 1.0" away from one of the connector halves, which was electrically grounded. Charge balanced current of 100 nA was run through this wire/connector for 1 hour per day to study skin growth. The other half of the connector "plug" floated with negligible electric current. The experiment was lost early and no conclusions were reached. The experiment is being repeated.

First results from the implanted flat cable are encouraging. Measurements taken on April 6th, thirteen days after implant, indicated less than 0.5 nA leakage on all five loops; 10 nA is the allowed upper limit. All five loops are intact.

The HMRI report on three implants completed during the Fall 1997 is included as Appendix I. The results are much as reported in the last quarterly report. The grooved Ti surface of TC-25 had significant dermal and subdermal growth, good ossiointegration and little or no infection. The smooth machined Ti surface of TC-26 had no significant skin growth and infection apparently spread down to the pedestal edge. The center of the pedestal showed no infection and no significant ossiointegration. TC-27 had 40 mils of Ta sponge material over the Ti dummy connector at skin level. Extensive infection occurred with no skin or bone integration. The HMRI report indicates that this was because the Ta did not promote skin attachment, but there were surgical difficulties because the hole cut in the skin was too large for the implant and had to be sutured to pull tight to the connector. Also, even if skin had grown it is likely that 40 mils of sponge Ta was too much. If skin grew five to ten mils into the material there would still be thirty or more mils of sponge providing a path for infectious organisms.

III. Change of Cable Geometry

A flat ribbon was fabricated by coating one mil Pt/Ir wires with 6 microns of Parylene. These wires were formed into five "loops" and placed side-by-side on tape. A Class 6 Silicone overcoat was applied with an overall width of less than 100 mils. After the top Silicone layer was partly cured the cable was lifted from the tape and coated on the bottom with Silicone. The wires were then welded to the bottom connector and the remainder of connector fabrication completed as usual with the cable being positioned in a ramp cut in the pedestal so it exited the connector at skull level, the cable laying flat on the skull below the temporalis muscle. The implant was uneventful and after three weeks appears intact with minimal leakage on all loops.

IV. Status of Fritting Experiment .

CABAL-12 preforms were expected by mid-March according to the last quarterly report. The tooling to fabricate the preforms has been difficult to make. The tool pins that form the holes through which wires will be placed to become connector pins break or deform when the preforms are made. The problems have been solved by tooling modifications and by using less pressure in forming the preforms. With less pressure it is possible that more shrinkage will occur at sintering, but that can be compensated by other means. At present preforms are expected by mid- to late-April and the first three parts with twelve pins of Mo, Pt/Ir and Ta are expected in late April. The purpose of using three pin types is to determine if there are problems with thermal effects. It is expected that the Pt/Ir may show problems in the form of cracked ceramic which would be unacceptable.

With the problems that have been experienced at least ten additional twelve pin dummy connectors will be fabricated prior to making tooling for the full 72 pin connectors. The first three of the dummys will be made with TA-23, another biocompatable ceramic with different properties than CABAL-12.

IV. Investigation Into Other Pin Matrix Materials

Work continues on finding alternatives to the glass/ceramic frit for long-term pin matrix material. A number of materials have been identified and more are expected. A thermal box has been constructed and the heater, temperature control and other components have been obtained. By mid-May this facility should be complete. Three Ti test fixtures have been ordered with 0.25" round holes into which the various material will be place with two Pt/Ir wires embedded for leakage testing. One side of the Ti plates will be covered with Silicone, the other will be open to the environment. These will be placed in Ringer's solution with one in the hot box at 80-90 °C and a second in the existing box at 37 °C for life testing.

V. Connection of Top and Bottom Sections with a "Quick Disconnect" Mechanism

The effort with Phoenix Interconnect to adapt an existing design to our smaller size failed. It was Phoenix Interconnect's opinion that it could not be done successfully.

A number of other designs are being developed in-house with prototype design to be finished by late May and prototypes fabricated in early Summer. The general nature of the designs being considered are a central snap, a circumferential snap (which would probably work better), a quarter turn (or less) design having a central pin or three "hooks" near the periphery, but internal. Other designs with less probability of success are also being considered including external rings with various catch mechanisms. This would increase the overall diameter of the external parts slightly, but not the percutaneous part. The larger external part may not be objectionable if the diameter does not grow to more than 0.75" since the patient could grasp it more easily than the smaller part. Consideration is being given in all designs to reducing the height of the external part to get a lower profile. This may be more critical than a slightly increased diameter.

VI. Reliability of the Anisotropic Material from Shin-Etsu

A sample of Shin-Etsu's GBM material has been obtained and an order for a quantity has been placed. Unlike the MAS material used in all earlier percutaneous work, the GBM material has Gold plated Brass pins spaced at regular intervals of 0.004" x 0.010". This is adequate to place the required number of pins in a 0.0142" diameter (AGW 27). The MAS material required use of AGW 25 wires as pins at 0.0179" to meet the elastomer specification. With this change it may be possible to increase the number of pins using the current geometry of an approximately 0.25" square. Ceramic material has been ordered to accommodate a 9x9 array of pins in the existing connector design. The first connector will be made with AWG27 Cu wire in May for connectivity testing of the GBM material. If this is successful then the door is open to move to the smaller pin diameter in all future work. It is also possible that this would be a key element in developing the quick disconnect if a central connection mechanism is required. The center nine pins could be replaced by the mechanism giving 72 pins.

The GBM material is being used in the HMRI implant with the new ribbon cable reported elsewhere. To date, no problems with the new elastomer have been reported.

The GBM material has a minimum thickness of 0.3 mm or 12 mils. The connector has been designed for an 0.2 mm elastomer. At present no change has been made to the connector because other modifications are expected before this summer and this change will be included at that time. The only change required will be the lengthening of the two upper connector screws by 12 mils at most, approximately one thread (0.1 mm is 4 mils).

VII. Connector Screws

Difficulties with the connector screw heads have been reported from two sources. The problem is that the Allen wrenches used to tighten the screws strip the hex hole in the screw head. In one case it is known that this occurred at 14 oz.-in of torque.

The screws presently in use have an "ultimate strength" of 50k psi. These are commercially pure grade 2. Alpha-beta alloy screws, grade 6AI-4V have a "ultimate strength" of 130k psi. These will be ordered for future work.

It would seem that the screw issue is not important since the screws will be replaced by the quick disconnect mechanism. That is true for the screws in the top connector section that must be tightened to 14 oz-in. However, similar screws will continue to be used in the lower connector and in the pedestal. It is prudent to replace these screws. Also, an accelerated implant program is anticipated in early Summer which will use the screws in the top connector and continued support of various research laboratories using the existing designs must continue.

VIII. Skin Growth

A meeting was held at the University of Washington in early February to explore the possibility of using the skin growth experience of UWEB (University of Washington Engineered Biomaterials) to improve the percutaneous results. UWEB reported success in determining the mechanism of Basal Cell growth and attachment. Three states of the Basal Cell have been determined, one of which is the mobile state adjacent to an injury. In this state the cell extends and secretes a precursor of Laminin-5, which modifies to Laminin-5 and attaches to other Basal cells which have secreted the Laminin-5 precursor. The technique UWEB developed involves the application of three layers of material to a ceramic or metallic material, the top of which is Laminin-5. As Basal Cells grow, they reach the implanted object, the percutaneous connector in this case, and attach to the Laminin-5 forming a biological seal against infection or leakage of body fluids.

UWEB has reported success in using their treatment on the Titanium bits supplied by PI Medical. It is expected that two dummy connectors will be prepared with Laminin-5 during the month of May for implant at HMRI.

There has been concern expressed about the strength of skin growth to a hard surface such as the Titanium. With soft tissue such as Basal Cells small motions could tear the tissue from the metal, at least in small areas. This would leave an opening even though the skin attachment was basically sound. To prevent this requires two steps. First, the dermal layer must also attach to the metal through Fibroblast and/or other connective tissue. The grooved Titanium encourages this attachment and earlier success using grooved Titanium suggests that if Basal Cells will attach there should be some underlying dermal support. Second, it would be desirable to have a "tougher" scar tissue adjacent to the metal to strengthen the abrupt tissue to metal interface. The force of skin or muscle movement would be dissipated in the scar tissue rather than propagating to the metal with excessive stress at the interface.

It is expected that the UWEB technique will provide the seal against infection that is needed for a successful skin attachment. This will be a significant step in skin growth and attachment work.

UWEB is funded by the National Science Foundation and PI Medical is an industrial member of the UWEB consortium.

IX. Activities for the First Quarter of 1998

During the next quarter:

- Reimplant the split electrostimulation experiment.
- Implant a full electrostimulation experiment.
- Implant two dummy connectors with the UWEB surface. The split UWEB experiment will not be run as planned earlier because of difficulties with the split experiment method.
- Implant a quick disconnect design.
- Increase pin count in the existing design by using AGW 27 at a 14.2 mils diameter. with the Shin-Etsu GBM anisotropic elastomer. The elastomer was used in one implant during the forth quarter.
- Start Parylene-Silicone interface accelerated life test.
- Start the polymer pin matrix life test.
- Coordination meeting will be held at HMRI during May.

Appendix I

HMRI Report

DEVELOPMENT OF A PERCUTANEOUS CONNECTOR SYSTEM

Contract #NO1-DC-7-2103

Quarterly Progress Report Number 4

January - March, 1998

Submitted to P.I. MEDICAL

Portland, OR

Submitted by

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Introduction

In this quarter, we present; 1) the results of TC-25 and TC-26, two animals that we briefly reported in our last QPR, and 2) the results from a third animal, TC-27, in which tantalum sponge was attached to the sides of the lower stage of the connector. Devices are currently under development that are expected to produce good biocompatibility and optimal base pedestal osseointegration with adequate soft tissue attachment to the lower stage and base pedestal after surgical implantation.

The rationale for the application of tantalum mesh sponge was to improve attachment of the skin and subcutaneous connective tissues to the connector by preventing marsupialization and preventing infection of the soft tissues immediately adjacent to the connector. However, in TC-27, the tantalum sponge produced the opposite effect. The metal sponge seemingly prevented, rather than improved, skin and soft tissue attachment to the connector. Electrical leak tests were not performed on any of these animals because dummy connectors were used. Histologic evaluations of the metal-bone interface and surrounding soft tissues were performed using light microscopy. In TC-27, pilot alkaline phosphatase (AP) cytochemistry and immunocytochemistry experiments were performed. AP activity was expressed within inflammatory cells and other stellate cells resembling osteoblasts. Studies with anti-CD antibodies to identify leukocyte subsets and ICAM-1, an adhesion molecule marker of blood vessel sprouting were not successful.

Methods

Percutaneous Connector Design. The percutaneous connector has been described in detail in previous QPR's. Briefly, the percutaneous connector consists of a base pedestal composed of titanium metal. The underside of the pedestal is composed of sintered titanium beads, and the sides have circumferential milled grooves, ca. 100 μm deep and 125 μm wide. The beads and grooves have been designed to facilitate the attachment of bone, skin and muscle tissues to the connector. The lower (percutaneous) stage contains the pin grid array, pin-wire bonds, silicone and epoxy potting surrounded by a titanium ring with circumferential grooves approximately 20 μm deep and 40 μm wide. The upper connector stage, i.e., the mating system, resides above the skin line. The design of the upper connector stage was slightly different in each of the three cats. The sides of the lower stages were machined finished with fine grooves ca. 50 μm wide. In TC-27, tantalum mesh sponge was added to the sides of the upper connector stage.

Surgical Procedures. Dummy connectors were implanted over the left hemisphere of each adult cat using general anesthesia and aseptic technique. A rainbow-type incision measuring approximately 4 cm was made on the right parietal hemisphere. The skin and muscles were retracted from the left hemisphere. A mark was made 16 mm distal to the cruciate sulcus and 9 mm from the midline at which point a central hole was drilled to accept the tip of the major drill, which was used to shape the skull to confirm to the bottom of the pedestal. The drill worked very well, and the pedestals fit the convexity satisfactorily. The retaining screws were inserted into the skull using a torque wrench. The pedestals appeared to be firmly attached to the skull. The lower stage and Teflon caps were added to the base pedestals . Then the muscles were sutured over the pedestal, and to the outer surface of the intact muscles on the right side. The galea was closed as

the second layer, bacitracin was added, and the skin wound was closed.

Vascular Perfusion, Embedding and Sectioning.

Eight weeks (TC-25, TC-26), or 9 weeks (TC-27) after surgical placement of the connectors, anesthetized animals were perfused with either PBS (TC-25, TC-26) or Ringer's Solution (TC-27) for blood removal followed by either ½ Karnovsky's Fixative in 0.1 M Schultz's Phosphate Buffer at pH: 7.3 (TC-25, TC-26), or with 4% formalin and 0.1% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH: 7.3 for enzyme cytochemistry (TC-27). After fixation, cat TC-27 was perfused with a mixture of sucrose and sodium cacodylate buffer (500 ml) and placed into a cold room at 6° C. All animals remained in a cold room for 1-3 days prior to removal of the connectors. Each connector and associated skin, muscle and bone was removed with a Lipshaw Bone Saw as a single tissue block (connector blocks). Skull areas distant from the connector were also removed to serve as control material (TC-27). The connector blocks were immersed into fresh fixative (according to the fixative type used in the original perfusion) for several additional hours. All connector blocks were embedded in glycol methacrylate and 20-40 μm sections were cut with a Beuhler Saw. Unpolished sections were mounted on glass slides with a rapid bonding adhesive and were stained with toluidine blue for light microscopy examination.

Cytochemistry and Immunochemistry.

The methods of Mayahara et al. (1967) and Wilson and Hodges (1979) were used for the demonstration of AP activity. These methods utilized the rich AP activity within cells including osteoblasts and inflammatory cells using alkaline lead citrate as the capturing agent for the available phosphate ions. A 30-40 μm section of the connector was trimmed down to 1.5 x 2 cm

using a diamond scribe, such that the soft tissue, bone and metal specimen glued to the glass slide would fit into a small incubation jar. After incubation at 37° C in a shaker water bath for 30 min, the sections were reacted with diluted ammonium sulfide solution to produce a dense lead sulfide precipitate (site of AP reactivity) that was visible microscopically. This section was lightly stained with toluidine blue and it was examined with the light microscope.

On another section, the skin and subcutaneous tissues with adjacent tantalum mesh and bone were trimmed away from the edges of the metal connector into 2x4 mm pieces. These glycol methacrylate-embedded tissue samples were incubated with monoclonal antibodies for the presence of ICAM-1, CD4, CD8 and CD13 according to previous preembedding methodology (Lossinsky et al., 1997; Lossinsky and Wisniewski, 1998).

Results

Autopsy Results (TC-25, TC-26, TC-27). Gross necroscopic evaluations of these three animals revealed minor variation in the placement of the implants on the skulls. Each connector was positioned on the left parietal skull lateral to the midline and adjacent to the left ear. The skin flaps adjacent to the ears were level with the cap of the connector. More medially, the skin levels were below the lower connector. In TC-26, the skin was retracted from the metal connector and dried encrusted exudate was present. In TC-27. The skin and soft tissues were retracted further from the metal connector, even more pronounced compared to TC-26. In TC-25, the skin was attached to the upper connector.

Histology. The findings were quite variable in the three animals. In TC-25, there was good attachment of the reticular layer of the dermis and subcutaneous skin layers to the sides of

both the lower and base pedestal stages of the connector Fig. 1). In skin samples from sites either adjacent to the connector, or in skin samples distant from the connectors, there was no evidence of infection. This conclusion was based on four representative cross-sections from different areas of the lower and pedestal stages. In TC-26, six cross-sectional views of the connector showed an active inflammatory process, both within the dermis and subcutaneous tissue at the sides of the relatively smooth, lower stage and extending down to the bony skull at the perimeter of the base pedestal (Figs. 2, 3). However, sections of plastic-embedded skin tissue, did not present evidence of infection. An occasional inflammatory cell was seen under the titanium base pedestal. Cells types within the inflammatory lesions included neutrophils, mononuclear cells and phagocytes. These were within all layers of the skin and subcutaneous tissue, suggesting a chronic inflammatory process. The dermis and subcutaneous tissue appeared to be firmly attachment to the grooved surface of the base pedestal (Fig. 2). There appeared to be recession of the epidermis and dermis away from the lower stage of the connector and little tissue was attached to its smooth surface. In TC-27, based on 10 sections examined, the epidermis, dermis and subcutaneous tissues were intermingled within the tantalum mesh sponge. Little soft tissue was seen attached to the lower stage or to the base pedestal (Fig. 4). Both acute (neutrophils) and chronic (mononuclear and macrophages) inflammatory cells were seen within the tantalum mesh and juxtaposed to both stages of the connector as well as underneath the connector (Figs. 5, 6). The interface between the skull and the base pedestal contained connective tissue cells primarily composed of fibroblasts and collagen bundles, mixed with neutrophils and large mononuclear phagocytes (Fig. 7).

Cytochemistry, Immunochemistry, Osseointegration.

In an attempt to examine the nature of osseointegration at the metal/bone interface, alkaline phosphatase (AP) cytochemistry was performed on one of the sections cut from TC-27. We found AP-positive cells, (presumably macrophages) among the conglomeration of cells within the tantalum mesh sponge network (Figs. 5, 6). Moreover, few stellate-shaped cells located within the connective tissue situated between the bone and metal were AP-positive. These cells located near the skull surface were presumed to be osteoblasts (Fig. 7). Immunoreactivity for ICAM-1 or any of the CD antigens was not present.

Osseointegration was variable in these three animals, shown in Table I. The width of the pedestal base-bone interface was measured in several histologic section (3-10 sections) using an ocular lens scale on an Olympus Light Microscope. Osseointegration was identified as osteoid tissue directly attached to the opaque titanium metal. Any evidence of blue stained, fibrotic subcutaneous connective tissue within the bone-metal interface was interpreted as the absence of osseointegration. The average amount of osseointegration in TC-25 was calculated as 66.3%, based on three representative regions (edges and the central portions of the connector) (Fig. 8). The amount of osseointegration in one central region of the pedestal from this animal was calculated to be 79%. In TC-25, TC-26, and also in one section from TC-27, excellent osseointegration was observed around the titanium screws inserted into the skull (Fig. 9). There was essentially no osseointegration between the base pedestal and bone in TC-26 and TC-27. The bone-metal interface indicated only fibrotic, connective and subcutaneous tissue, including stellate fibroblasts and some inflammatory cells described above (Figs. 7, 10).

Table I

ANIMAL #	LOCATION OF INFLAMMATION	AVERAGE OSSEOINTEGRATION
TC-25	None	66.3%
TC-26	Pedestal sides within the dermis and subcutaneous tissue extending to the periphery of the pedestal-bone interface	1.17%
TC-27	1).Pedestal sides within the dermis and subcutaneous tissue; 2) within the tantalum sponge; 3) within the pedestal-bone interface	< 1%

Discussion

Based on the results of three animals in this quarter, we present the following observations:

1) Skin Attachment to the Metal Connector. Our results suggest that the dermis and subcutaneous portions of the skin appeared to attach better to grooved titanium surfaces than to a semi-smooth surface. This was to be expected considering that the stratum corneum layer of the epidermis in the cat is composed of a thick avascular layer of keratinized cells at its surface with numerous hair follicle shafts. Consequently, the outer layer of the skin continuously receives oils released from sweat and sebaceous glands located within the reticular layer of the dermis. Considering the composition of the desquaminating epidermal layer in the cat, it is likely that the smoother, machined surface of the lower stage of the connector may not optimally support tissue attachment. In contrast, the deeply grooved surface of the base pedestal demonstrated improved

attachment of the dermis and subcutaneous tissue that appeared healthy (rich in blood vessels and connective tissue, including fibroblasts that promote fibrosis and tissue adhesion). If the positions of the grooved and machined surfaces were reversed, ie., the grooves were made in the lower stage of the connector adjacent to the epidermis, and the smoother machined surface were on the sides of the base pedestal, we could further test the hypothesis that a grooved metal surface promotes better tissue attachment. This experiment is now in progress in our laboratory (TC-28).

We were surprised by poor results from TC27, with the tantalum mesh sponge around the outside of the connector. Tantalum is known to enhance soft tissue attachment to metal surfaces. Although we expected improved skin and soft tissue attachment to the titanium connector, what we found, in fact, was that the tantalum had the opposite effect in the cat, presumably due its skin composition mentioned above.

2) Osseointegration and Possible Affects of Infection. The question concerning chronic skin infection is important, especially as it relates to osseointegration. Although our results are limited to only three animals, they do suggest that skin infection may inhibit osseointegration. This conclusion is based on the severe infection observed within the bone-metal interface in both TC-26 and TC-27. In the animal in which 66.3% of the pedestal was osseointegrated, skin infection was not present, whereas, in animals with skin infections, little osseointegration occurred, with the exception of the excellent osseointegrastion around the bone screws in TC-26, and TC-27. Although this question is important, it remains unanswered at present because our previous experiments implied that infection may not have an influence on the degree of osseointegration. We based this initial conclusion on the fact that we noticed good osseointegration into titanium base pedestals in several animals despite inflammatory cells being

present within both the dermis and subcutaneous tissues (and also to a limited degree within the metal-bone interface). Since we noticed neutrophils and mononuclear cells near the edge of the base connector stages and the skulls in TC-26 and TC-27, it is not unreasonable to consider that downgrowth of the skin, concomitant with infection by bacterial and/or mycotic organisms may have had a deleterious effect on osseointegration. Leukocytes secrete pro-inflammatory cytokines and a host of other substances including hydrolytic enzymes, known to be harmful to cells. For this reason, their presence adjacent to the connector and especially within the metal-bone interface, supports the argument that local inflammatory cells could likely have a deleterious effect on osseointegration. It seems reasonable that the degree of osseointegration may be directly related to the amount of inflammatory cells present in the locally infected tissue. Perhaps increased numbers of inflammatory cells may contribute to the failure of osseointegration since it is likely that more hydrolytic enzymes would be excreted locally. We will pay close attention to this important question in future animals.

In TC-25, good skin attachment appeared to be one of several factors that permitted adequate osseointegration. For example, variability across animals produced by grinding into the skulls prior to fitting and attachment of the base pedestals may also explain differences in osseointegration. This premise is supported by the increased osseointegration observed in the sections from the center of the connectors from TC-25 and in several animals previously reported compared to decreased osseointegration in sections from the periphery of the connectors.

3) Cytochemistry and Immunochemistry. In TC-27, the presence of AP reaction product suggests the presence of osteoblasts within the fibrotic tissue of the metal-bone interface despite the apparent infiltration of immunocytes. This conclusion is based on the fact that osteoblasts are

rich in this enzyme and would be expected to be expressing this enzyme within the bone-metal interface. The presence of AP-positive immunocytes is also not unusual since leukocytes, especially active macrophages synthesize AP in addition to a spectrum of hydrolytic enzymes, which is part and parcel of their function in the inflammatory process (Hayhoe and Quaglino, 1958). The fact that ICAM-1 and the CD antigens were not expressed in the same animal is surprising. The incubation temperature for all primary antibodies was 4-8° C overnight, while the incubation temperature for the AP reaction was 37° C for ca. 1 hr. Thus, it is possible that the immunoreagents did not penetrate through the plastic during the overnight incubation at low temperature.

Future Studies

In future animals, we will continue light microscopic studies to determine if the skin and subcutaneous tissues attach differently to grooved and machined metal surfaces. In the most recent experiment, the design of the lower stage connector has been modified such that one half of the surface of its side was coated with sintered titanium beads, while the other half was uncoated, and has a smoother, machined surface. One connector of this type has been implanted (TC-28). It will be interesting to study the histology in this experiment with this "built in control". 2) We will correlate the degree of osseointegration with any obvious skin infection. 3) We will also attempt to develop methods to prevent skin marsupialization and infection along the connector base pedestal using prophylactic antibiotics The application of adding fibroblastic growth factor to the skin adjacent to the connector may improve the attachment of skin and soft tissue to the connector. We would like to apply immunocytochemical methods to examine bone-associated

enzymes, including alkaline phosphatase (osteoblast-specific), and acid phosphatase (osteoclast-specific) to identify possible changes in these enzymes near the titanium-bone interface. We also plan to investigate whether blood vessel-associated adhesion molecules may be related to osseointegration. The application of immunocytochemical approaches to identify leukocyte subsets will also be explored further. We will need to know what inflammatory cell subsets are present within the skin infection that often spreads to the metal-bone interface. Because anti-cat antibodies prepared against specific leukocyte antigens are available commercially, we may be able to identify the subsets of leukocytes in question. Blocking leukocyte receptors using antibodies against specific CD antigens (Simpson et al., 1988) or endothelial cell receptors with anti-adhesion molecule antibodies such as ICAM-1/CD54 and PACEM-1/CD31 have proven successful in inhibiting certain disease states by suppressing or blocking inflammatory cell influx (Archelos et al., 1993; Muller et al., 1993).

Although glycol methacrylate embedding method appears to be adequate for cytochemical reaction, our pilot immunocytochemical experiment failed to demonstrate the presence of either ICAM-1 or CD antigens in tissue that demonstrated positive AP reactivity. This is an indication that we may need to increase the temperature of immunoincubations for ICAM-1 and several CD antibodies from 4° C to 37° C. We will also continue testing tissue material from TC-27 in order to determine if the glycol methacrylate embedding material will be adequate for future immunocytochmical studies or whether we may need to switch to an alternative embedding material.

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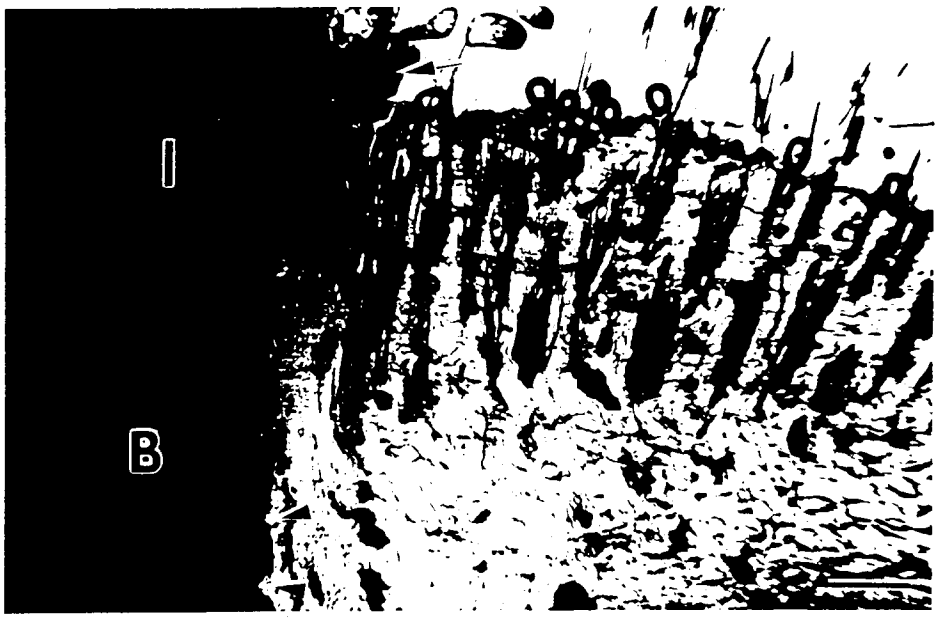

Fig. 1. TC-25. The epidermis (arrow) is attached to the edges of the lower stage (1) and base pedestal (B) shows good dermis and subcutaneous attachment to the grooved connector surfaces (arrowheads). Bar = 500μm.

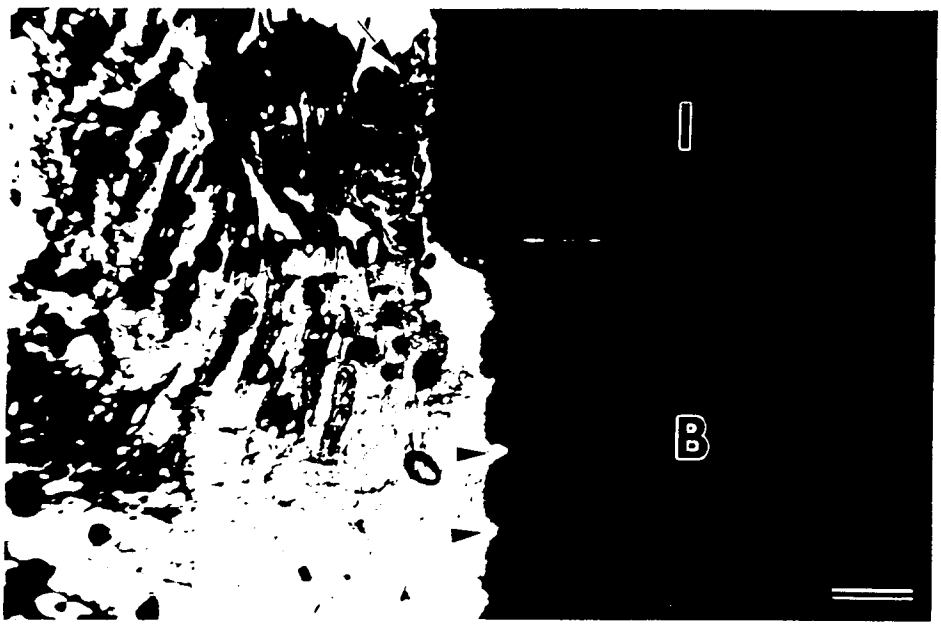

Fig. 2. TC-26 showed variable epidermal attachment (arrow) to the lower connector stage (1) in several sections and good attachment of the dermis and subcutaneous tissue to the grooves in the base pedestal (arrowheads). Bar = 500μm.

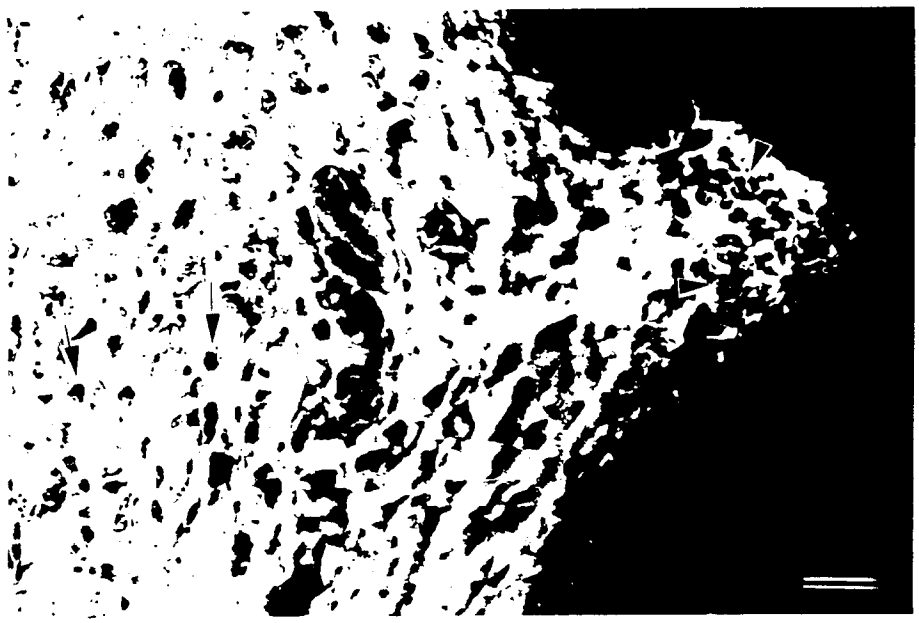

Fig. 3. TC-26 showed inflammatory cells within several layers of the skin and subcutaneous tissue. Neutrophils (arrowheads), and small lymphocytes (arrows) are shown within the grooves of the base pedestal and within the subcutaneous tissue respectively. The granular material represents titanium filings that entered the plastic during the cutting process. The focus is variable in this 40 μm section. Bar = 25μm.

Fig. 4a. TC-27. The edge of the lower stage of the connector (1) is shown. Note the pieces of the tantalum mesh sponge (arrowheads) and the poor dermis and subcutaneous attachment to the connector (*) Bar = 200μm.

Fig. 4b. TC-27. The bottom edge of the lower stage (1) and top edge of the base pedestal (B) are shown. Within the tantalum mesh, small granular cells are stained with AP reaction product (arrowheads), also seen at higher magnification in Fig. 5. Bar = 200μm.

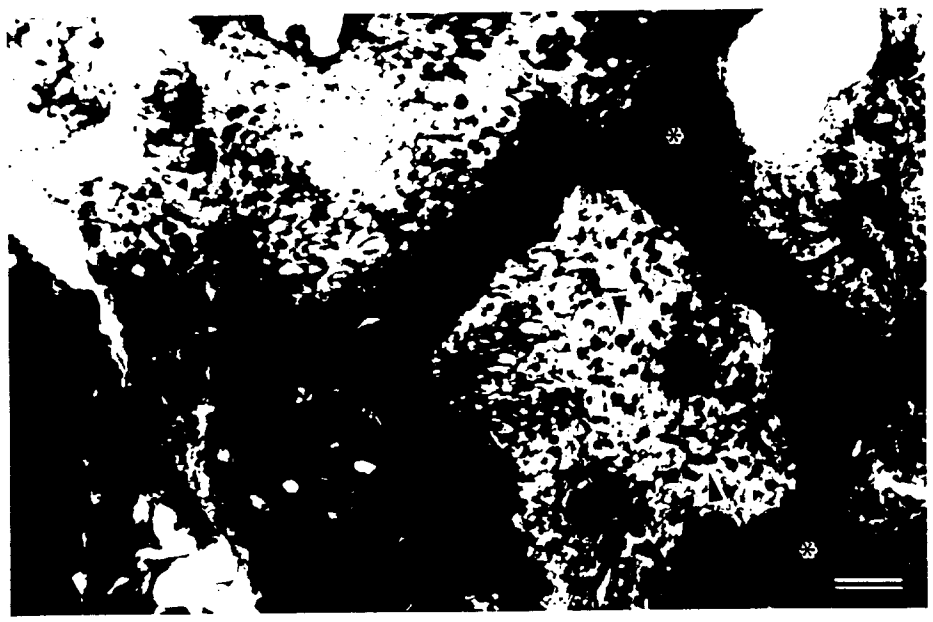

Fig. 5. TC-27. Higher magnification of the section in Figure 4b shows mononuclear cells within the tantalum mesh sponge (*) stained with AP reaction product (arrowheads). Bar = 100μm.

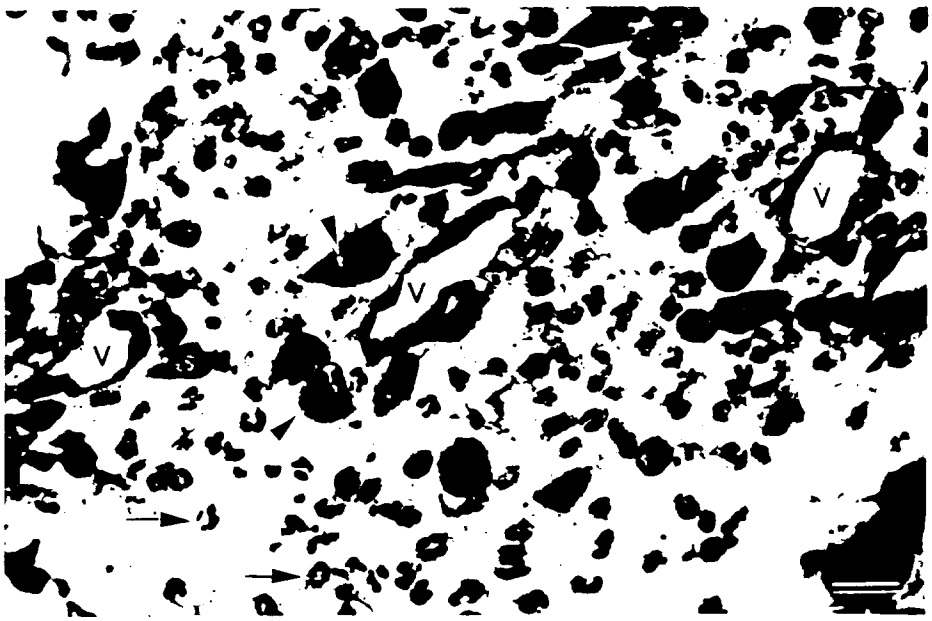

Fig. 6. TC-27. AP-positive cells (arrowheads) are seen within the metal-bone interface together with numerous neutrophils (arrows). Although not clearly shown in this thick-section, capillaries also stained positive for AP (v). This mixture of mononuclear cells and neutrophils is an indication that there is a constant release of pro-inflammatory cytokines from the mononuclear cells that in tern, recruit more neutrophils, similar to an acute inflammatory response. Bar = 100μm.

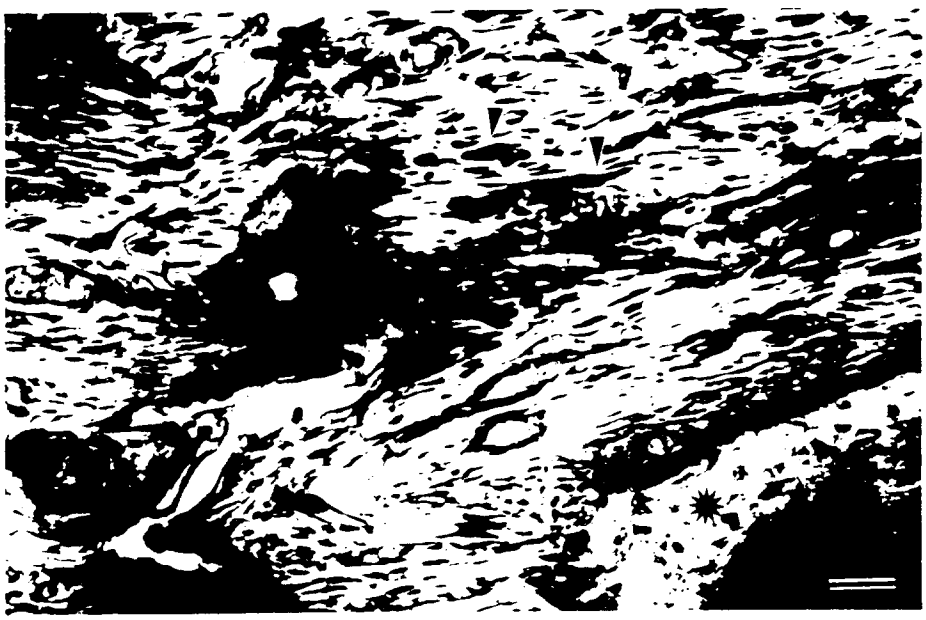

Fig. 7. TC-27, AP reaction. Several stellate cells (arrowheads) within the metal-bone interface stained a brown color. These cells were positioned adjacent to the bone (*) suggesting that they may be newly formed osteoblasts. Bar = 50μm.

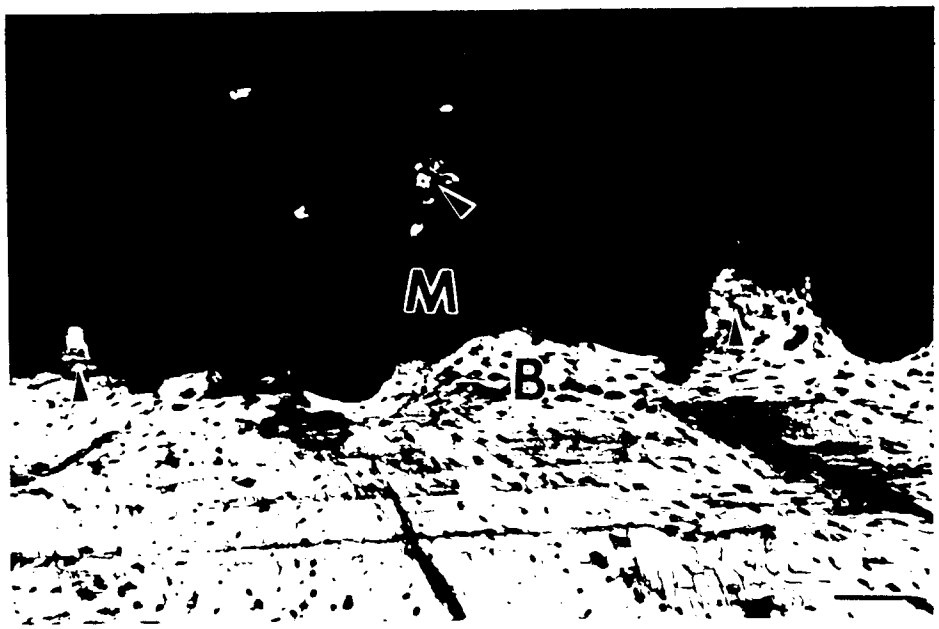

Fig. 8. TC-25. The interface between metal (M) and bone (B) of the base pedestal is shown. Note the presence of osteoid tissue within and around the sintered titanium beads (arrowheads). Bar = 100μm.

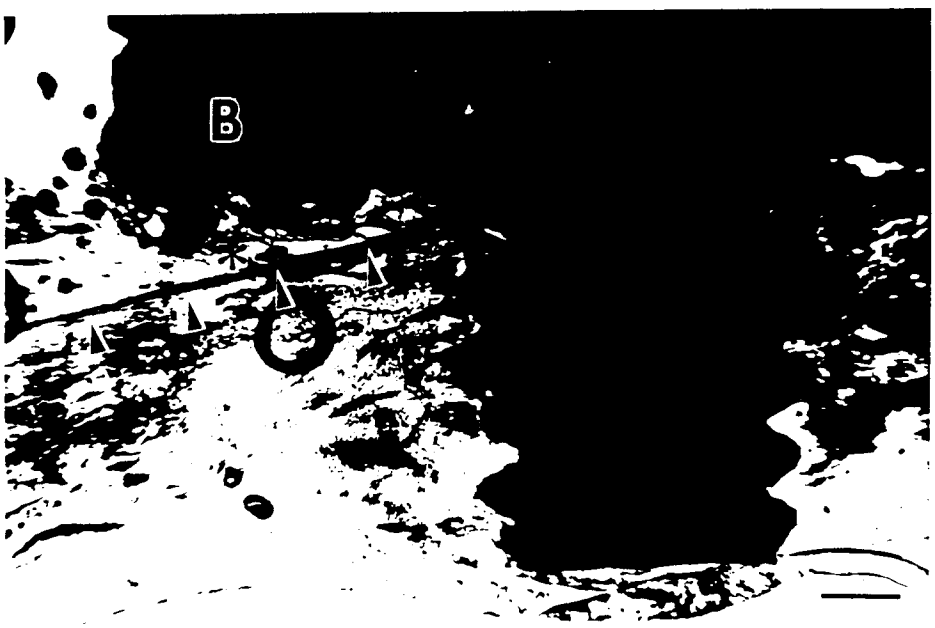

Fig. 9. TC-26. Good osseointegration around the titanium bone screws is shown. Note the boundary between the base pedestal (B) and the skull (arrowheads). The subcutaneous connective tissue has grown under the base pedestal (*), but is not well shown in this micrograph. Bar = 500μm.

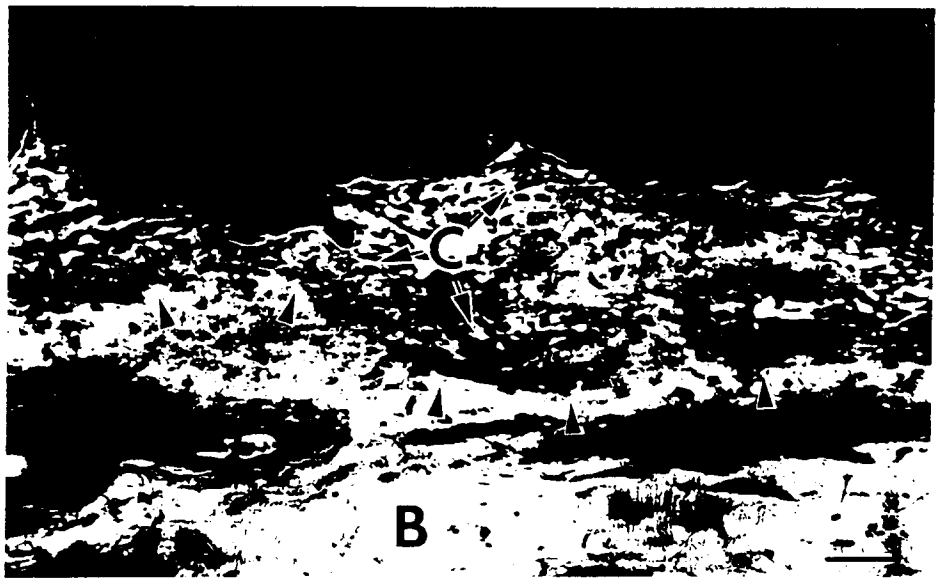

Fig. 10. TC-26. This section is closer to the center of the base pedestal. The gap between the bone (demarcated with arrowheads) and the opaque titanium metal is filled with connective tissue (C→). Bar = 50μm.